This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representation of
The original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

**As rescanning documents *will not* correct images,
please do not report the images to the
Image Problem Mailbox.**

Paul

BEST AVAILABLE COPY

Access DB# 77739

SEARCH REQUEST FORM

Scientific and Technical Information Center

Requester's Full Name: Phyllis Spivack Examiner #: 70400 Date: 10/8/02
Art Unit: 1614 Phone Number 308 4703 Serial Number: 09/955485
Mail Box and Bldg/Room Location: 7D05 Results Format Preferred (circle): PAPER DISK E-MAIL

If more than one search is submitted, please prioritize searches in order of need.

****

Please provide a detailed statement of the search topic, and describe as specifically as possible the subject matter to be searched. Include the elected species or structures, keywords, synonyms, acronyms, and registry numbers, and combine with the concept or utility of the invention. Define any terms that may have a special meaning. Give examples or relevant citations, authors, etc, if known. Please attach a copy of the cover sheet, pertinent claims, and abstract.

Title of Invention: ↑ Cerebral Bioavailability of Drugs

Inventors (please provide full names): Michael A. Moskowitz
James K. Liao

Earliest Priority Filing Date: 3/19/99

**For Sequence Searches Only* Please include all pertinent information (parent, child, divisional, or issued patent numbers) along with the appropriate serial number.*

Please search
methods of increasing cerebral bioavailability of a drug (NOT NO) (any)
comprising administering a NO-increasing agent, such
as L-arginine, NADPH, tetrahydrobiopterin,
optionally, wherein the increase in NO is
through preexisting ecNOS. L18

start c #22

Thanks.

POINT OF CONTACT:
PAUL SCHULWITZ
TECHNICAL INFO. SPECIALIST
CM1 6B06 TEL. (703) 305-1954

****

STAFF USE ONLY

Searcher: _____
Searcher Phone #: _____
Searcher Location: _____
Date Searcher Picked Up: 10/9
Date Completed: 10/10
Searcher Prep & Review Time: 60
Clerical Prep Time: _____
Online Time: 108

Type of Search

NA Sequence (#)_____
AA Sequence (#)_____
Structure (#) _____
Bibliographic _____
Litigation _____
Fulltext X
Patent Family _____
Other _____

Vendors and cost where applicable

STN 300.46
Dialog _____
Questel/Orbit _____
Dr.Link _____
Lexis/Nexis_____
Sequence Systems _____
WWW/Internet _____
Other (specify)_____

PTO-1590 (8-01)

ER 37 OF 2229 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1999:194308 BIOSIS
DN PREV199900194308
TI Normal and pathological distribution of nitric oxide in the cardiovascular system.
AU Malinski, Tadeusz (1)
CS (1) Center for Biomedical Research, Oakland University, Rochester, MI, 48309 USA
SO Polish Journal of Pharmacology, (Nov.-Dec., 1998) Vol. 50, No. 6, pp. 387-391.
ISSN: 1230-6002.
DT Article
LA English
AB Using microsensors, it is possible to quantify the amount and concentration of nitric oxide (NO) release throughout the cardiovascular system in veins, arteries and the heart. Under normal physiological conditions a well defined distribution of NO is maintained. This concentration depends on the laminar, turbulent, or pulsatile flow rate of blood. Significantly reduced production of NO is observed in the pathogenesis of cardiovascular disorders like hypertension, atherosclerosis and diabetes. This is due to increased generation of superoxide by a dysfunctional endothelium and the rapid formation of peroxynitrite followed by formation of peroxynitrite followed by the formation of highly reactive OH and NO2 radicals and NO2+. Elevated concentration or improved mass transport of L-**arginine** and (6)-5,6,7,8-**tetrahydrobiopterin** can be applied to increase/decrease NO/superoxide release by the dysfunctional endothelium.
AB. . . followed by the formation of highly reactive OH and NO2 radicals and NO2+. Elevated concentration or improved mass transport of L-**arginine** and (6)-5,6,7,8-**tetrahydrobiopterin** can be applied to increase/decrease NO/superoxide release by the dysfunctional endothelium.
IT . . .
atherosclerosis: pathogenesis, vascular disease; diabetes: endocrine disease/pancreas, vascular disease, pathogenesis, metabolic disease; hypertension: pathogenesis, vascular disease
IT Chemicals & Biochemicals
(6)-5,6,7,8-**tetrahydrobiopterin**; nitric oxide: cardiovascular, normal distribution, pathological distribution, pathogenic role; L-**arginine**
IT Alternate Indexing
Atherosclerosis (MeSH); Diabetes Mellitus (MeSH); Hypertension (MeSH)
RN 10102-43-9 (NITRIC OXIDE)
74-79-3 (L-**ARGININE**)

L6 ANSWER 45 OF 2229 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1999:56971 BIOSIS
DN PREV199900056971
TI Anti-pterins as tools to characterize the function of **tetrahydrobiopterin** in NO synthase.
AU Boemmel, Heike M.; Reif, Andreas; Froehlich, Lothar G.; Frey, Armin; Hofmann, Heinrich; Marecak, Dale M.; Groehn, Viola; Kotsonis, Peter; La, Mylinh; Koester, Sandra; Meinecke, Matthias; Bernhardt, Manfred; Weeger, Monika; Ghisla, Sandro; Prestwich, Glenn D.; Pfleiderer, Wolfgang; Schmidt, Harald H. H. W. (1)
CS (1) Dep. Pharmacol. Toxicol., Julius-Maximilians-Univ., Versbacher Strasse 9, D-97078 Wuerzburg Germany
SO Journal of Biological Chemistry, (Dec. 11, 1998) Vol. 273, No. 50, pp. 33142-33149.
ISSN: 0021-9258.
DT Article
LA English
AB Nitric oxide synthases (NOS) are homodimeric enzymes that NADPH-dependently convert L-**arginine** to nitric oxide and L-citrulline. Interestingly, all NOS also require (6R)-5,6,7,8-tetrahydro-L-biopterin (H4Bip) for maximal activity although the mechanism is not fully understood. Basal NOS activity, i.e. that in the absence of exogenous H4Bip, has been attributed to enzyme-associated H4Bip. To elucidate further H4Bip function in purified NOS, we developed two types of pterin-based NOS inhibitors, termed anti-pterins. In contrast to type II anti-pterins, type I anti-pterins specifically displaced enzyme-associated H4Bip and inhibited H4Bip-stimulated NOS activity in a fully competitive manner but, surprisingly, had no effect on basal NOS activity. Moreover, for a number of different NOS preparations basal activity (percent of Vmax) was frequently higher than the percentage of pterin saturation and was not affected by preincubation of enzyme with H4Bip. Thus, basal NOS activity appeared to be independent of enzyme-associated H4Bip. The lack of intrinsic 4alpha-pterincarbinolamine dehydratase activity argued against classical H4Bip redox cycling in NOS. Rather, H4Bip was required for both maximal activity and stability of NOS by binding to the oxygenase/dimerization domain and preventing monomerization and inactivation during L-**arginine** turnover. Since anti-pterins were also effective in intact cells, they may become useful in modulating states of pathologically high nitric oxide formation.
TI Anti-pterins as tools to characterize the function of **tetrahydrobiopterin** in NO synthase.
AB Nitric oxide synthases (NOS) are homodimeric enzymes that NADPH-dependently convert L-**arginine** to nitric oxide and L-citrulline. Interestingly, all NOS also require (6R)-5,6,7,8-tetrahydro-L-biopterin (H4Bip) for maximal activity although the mechanism is not. . . for both maximal activity and stability of NOS by binding to the oxygenase/dimerization domain and preventing monomerization and inactivation during L-**arginine** turnover. Since anti-pterins were also effective in intact cells, they may become useful in modulating states of pathologically high nitric. . .
IT . . . Concepts
Enzymology (Biochemistry and Molecular Biophysics); Methods and Techniques
IT Chemicals & Biochemicals
anti-pterins: chemical tool; nitric oxide synthase [NOS]; **tetrahydrobiopterin**: functional characterization; tritiated-PHS-176: photoaffinity label; L-**arginine**
IT . . .
Systems S-tagged domain purification protocol: Isolation/Purification Techniques: CB, purification method; 2',5'-ADP-Sepharose affinity chromatography: affinity chromatography, purification method
IT Miscellaneous Descriptors
L-**arginine** turnover
RN 2236-60-4D (PTERINS)
17528-72-2 (TETRAHYDROBIOPTERIN)
125978-95-2 (NO SYNTHASE)

125978-95-2 (NITRIC OXIDE SYNTHASE)
74-79-3 (L-**ARGININE**)
7783-20-2 (AMMONIUM SULFATE)
10102-43-9 (NITRIC OXIDE)
2236-60-4 (PTERIN)

L6 ANSWER 46 OF 2229 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1999:49154 BIOSIS
DN PREV199900049154
TI Induction of inducible nitric oxide synthase and its corresponding **tetrahydrobiopterin**-cofactor-synthesizing enzyme GTP-cyclohydrolase I during cutaneous wound repair.
AU Frank, Stefan (1); Madlener, Marianne; Pfeilschifter, Joset; Werner, Sabine
CS (1) Institut fuer Allgemeine Pharmakologie und Toxikologie, Klinikum der JWG-Universitaet Frankfurt/M., Theodor-Stern-Kai 7, D-60590 Frankfurt/M. Germany
SO Journal of Investigative Dermatology, (Dec., 1998) Vol. 111, No. 6, pp. 1058-1064.
ISSN: 0022-202X.
DT Article
LA English
AB Recent work has suggested a possible role of nitric oxide, a free radical gas, during the wound healing process. In this study we investigated the regulation of inducible nitric oxide synthase (iNOS) and GTP-cyclohydrolase I (GTP-CH I), the rate-limiting enzyme in the biosynthesis of the iNOS cofactor (6R) 5,6,7,8-**tetrahydrobiopterin** (6-BH4), during the repair process. We found a similar time course of induction of iNOS and GTP-CH I expression, whereas absolute expression levels were different for both genes. Immunohistochemical analysis revealed colocalization of iNOS and GTP-CH I proteins in the wound. Systemic treatment with glucocorticoids significantly altered the expression levels of iNOS and GTP-CH I. Expression of iNOS and GTP-CH I was suppressed by glucocorticoids in normal, and to a much greater extent in wounded skin. Furthermore, a role of nitric oxide as a novel mediator of gene regulation during healing is suggested by the demonstration of nitric oxide-mediated induction of vascular endothelial growth factor expression in keratinocytes. These findings may provide an explanation for the beneficial effects of orally supplemented L-**arginine** on wound healing, and suggest that a disturbed induction of iNOS and GTP-CH I expression may at least partially underlie the wound healing defect seen in glucocorticoid-treated animals.
TI Induction of inducible nitric oxide synthase and its corresponding **tetrahydrobiopterin**-cofactor-synthesizing enzyme GTP-cyclohydrolase I during cutaneous wound repair.
AB. . . nitric oxide synthase (iNOS) and GTP-cyclohydrolase I (GTP-CH I), the rate-limiting enzyme in the biosynthesis of the iNOS cofactor (6R) 5,6,7,8-**tetrahydrobiopterin** (6-BH4), during the repair process. We found a similar time course of induction of iNOS and GTP-CH I expression, whereas. . . vascular endothelial growth factor expression in keratinocytes. These findings may provide an explanation for the beneficial effects of orally supplemented L-**arginine** on wound healing, and suggest that a disturbed induction of iNOS and GTP-CH I expression may at least partially underlie. . .
IT . . .
Diseases
wound healing defect: integumentary system disease
IT Chemicals & Biochemicals
glucocorticoid; inducible nitric oxide synthase: induction; GTP-cyclohydrolase I: expression, **tetrahydrobiopterin**-cofactor-synthesizing enzyme; L-**arginine**
RN 125978-95-2 (NITRIC OXIDE SYNTHASE)
37289-19-3 (GTP-CYCLOHYDROLASE I)
74-79-3 (L-**ARGININE**)

L6 ANSWER 47 OF 2229 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1999:48165 BIOSIS

DN PREV199900048165
TI Biosynthesis of NO: Mechanism, regulation and control.
AU Sennequier, Nicolas; Goff, Sandrine Vadon-Le (1)
CS (1) CNRS URA 400, 45 rue des Saints-Peres, 75270 Paris Cedex 06 France
SO M-S (Medecine Sciences), (Nov., 1998) Vol. 14, No. 11, pp. 1185-1195.
ISSN: 0767-0974.
DT General Review
LA French
SL French; English
AB Nitric oxide (NO), a reactive molecule, is a biological mediator synthesized by the three isoforms of NO synthase (NOS), two of which are constitutive (NOS-1 and NOS-3), and one inducible (NOS-2). These homodimeric heme enzymes catalyze the oxidation of their substrate, L-**arginine**, in the presence of NADPH, molecular oxygen and **tetrahydrobiopterin**, into a hydroxylated intermediate, NOHA, and then into citrulline and NO. The heme is probably responsible for both steps of product formation. The C-terminal half of NOS has a sequence homology with cytochrome P450 reductase. In the N-terminal half, where substrate oxidation is carried out, comparison to P450 shows the conservation of several amino-acids surrounding the cysteine responsible of heme coordination. NOS is therefore an autonomous P450 system. Furthermore, the dimeric structure of NOS-2 is essential for its activity, potentially because it is crucial to re constitution of the active site. Two recent crystal structures of NOS-2 (monomer and dimer) show unique features in NOS structure. Oxidation of NOHA into NO by NOS is an atypical monooxygenation because it requires only a half-equivalent of NADPH. NOS-mediated NOHA oxidation into citrulline and NO might be carried out in a unique mechanism by an iron peroxide resulting from molecular oxygen binding to NOSFe(II). The NOS are regulated in a number of ways, including transcriptionally (especially NOS-2), by calcium/calmodulin binding (for the constitutive isoforms), by some of their cofactors, and by their substrates and products. At low levels, NO seems involved in the transmission of information, especially in blood pressure regulation, as a vasodilator, and in the nervous system. NO production at higher doses plays a role in immune response, through its cytostatic and cytotoxic properties, but also in several pathologies, including septic shock. As attempted treatments of the latter have shown, the selectivity of NOS inhibition is crucial to its therapeutic efficacy. Beyond action on its characteristics that are shared by other enzymes, which would therefore lack selectivity, selective NOS inhibition could be obtained by competitive substrate binding inhibitors, like S-alkylisothioureas or Nomega-propylarginine.
AB. . . which are constitutive (NOS-1 and NOS-3), and one inducible (NOS-2). These homodimeric heme enzymes catalyze the oxidation of their substrate, L-**arginine**, in the presence of NADPH, molecular oxygen and **tetrahydrobiopterin**, into a hydroxylated intermediate, NOHA, and then into citrulline and NO. The heme is probably responsible for both steps of. . .

29 BIOSIS COPYRIGHT 2002 BIOLOGI[redacted] ABSTRACTS INC.
AN 1999:35321 BIOSIS
DN PREV199900035321
TI **Tetrahydrobiopterin**, cytokines, and nitric oxide synthesis.
AU Werner, Ernst R. (1); Werner-Felmayer, Gabriele; Mayer, Bernd
CS (1) Inst. Med. Chem. Biochem., Univ. Innsbruck, Fritz-Pregl-Str. 3, A-6020 Innsbruck Austria
SO Proceedings of the Society for Experimental Biology and Medicine, (Dec., 1998) Vol. 219, No. 3, pp. 171-182.
ISSN: 0037-9727.
DT General Review
LA English
AB Nitric oxide synthases require a surprisingly rich selection of cofactors to perform the conversion of L-**arginine** to citrulline and nitric oxide (NO): NADPH, FAD, FMN, heme and **tetrahydrobiopterin**. In a previous minireview in this journal we summarized work concerning the induction of **tetrahydrobiopterin** biosynthesis by cytokines, which yields increased intracellular tetrahydroblopterin concentrations supporting NO formation by intact cells (P.S.E.B.M. 203:1-12). The present review updates work on the induction of **tetrahydrobiopterin** biosynthesis by cytokines, and summarizes recent advances in research of **tetrahydrobiopterin** dependence of the NO synthase reaction. Studies using recombinant NO syntheses and site-directed mutations thereof have localized several amino acids critical for **tetrahydrobiopterin** binding, which are discussed in reference to the recently published crystal structure of the dimer of the oxygenase domain of murine inducible NO synthase with substrate and pterin. Allosteric actions of **tetrahydrobiopterin** on NO synthases are stabilization of dimers, stabilization of a conformation with high-spin heme iron, and support of binding of the substrate L-**arginine**. Since the 4-amino analog of tetrahydroblopterin, which is a dihydropteridine reductase inhibitor, supports these allosteric actions but inhibits the enzyme activity, **tetrahydrobiopterin** appears to play a redox-active role in stimulating the NO synthase reaction in addition to its allosteric actions on NO synthases. Amelioration of endothelial dysfunction by **tetrahydrobiopterin** in animal models and in humans in vivo has been observed. It remains to be investigated, however, to what extent the role of **tetrahydrobiopterin** as cofactor of NO synthases contributes to these in vivo effects of **tetrahydrobiopterin**.

TI **Tetrahydrobiopterin**, cytokines, and nitric oxide synthesis.
AB Nitric oxide synthases require a surprisingly rich selection of cofactors to perform the conversion of L-**arginine** to citrulline and nitric oxide (NO): NADPH, FAD, FMN, heme and **tetrahydrobiopterin**. In a previous minireview in this journal we summarized work concerning the induction of **tetrahydrobiopterin** biosynthesis by cytokines, which yields increased intracellular tetrahydroblopterin concentrations supporting NO formation by intact cells (P.S.E.B.M. 203:1-12). The present review updates work on the induction of **tetrahydrobiopterin** biosynthesis by cytokines, and summarizes recent advances in research of **tetrahydrobiopterin** dependence of the NO synthase reaction. Studies using recombinant NO syntheses and site-directed mutations thereof have localized several amino acids critical for **tetrahydrobiopterin** binding, which are discussed in reference to the recently published crystal structure of the dimer of the oxygenase domain of murine inducible NO synthase with substrate and pterin. Allosteric actions of **tetrahydrobiopterin** on NO synthases are stabilization of dimers, stabilization of a conformation with high-spin heme iron, and support of binding of the substrate L-**arginine**. Since the 4-amino analog of tetrahydroblopterin, which is a dihydropteridine reductase inhibitor, supports these allosteric actions but inhibits the enzyme activity, **tetrahydrobiopterin** appears to play a redox-active role in stimulating the NO synthase reaction in addition to its allosteric actions on NO synthases. Amelioration of endothelial dysfunction by **tetrahydrobiopterin** in animal models and in humans in vivo has been observed. It remains to be investigated, however, to what extent the role of **tetrahydrobiopterin** as

cofactor of NO synthases contributes to these in vivo effects of **tetrahydrobiopterin**.

IT Major Concepts
Biochemistry and Molecular Biophysics

IT Chemicals & Biochemicals
cytokines; nitric oxide synthase; nitric oxide: synthesis; **tetrahydrobiopterin**: biosynthesis, enzyme cofactor

RN **17528-72-2** (**TETRAHYDROBIOPTERIN**)
10102-43-9 (NITRIC OXIDE)
125978-95-2 (NITRIC OXIDE SYNTHASE)

ACCESSION NUMBER: 1997:348652 BIOSIS
DOCUMENT NUMBER: PREV[redacted]799647855
TITLE: Interactions between **nitric oxide** and dopamine in inhibitory learning and memory in newborn rate.
AUTHOR(S): Myslivecek, J. (1); Barcal, J.; Hassmannova, J.; Zahlava, J.; Zalud, V.
CORPORATE SOURCE: (1) Inst. Pathophysiol., Charles Univ., Med. Fac. Plzen, CZ-301 66 Plzen Czech Republic
SOURCE: Neuroscience, (1997) Vol. 79, No. 3, pp. 659-669. ISSN: 0306-4522.
DOCUMENT TYPE: Article
LANGUAGE: English

AB Taking into account our previous results on dopamine and **nitric oxide** effects on neonatal inhibitory learning and memory in rats, the mutual interactions of the two molecules were studied in this experimental paradigm. Both **increased** dopamine content and **nitric oxide bioavailability** in the **brain** after application of dopamine and L-**arginine** as substrate for **nitric oxide** synthase solutions into lateral cerebral ventricles improved learning and 24 h memory. Joint application of dopamine and L-**arginine** yielded still more improvement. Learning and memory processing were dose dependently enhanced by D-1 receptor agonists as well, whereas D-1 receptor antagonists had an opposite and also dose-dependent effect. Dopamine or D-1 receptor agonists administered together with nitro-L-**arginine**, a **nitric oxide** synthase inhibitor that impaired learning and memory due to a decreased **nitric oxide** availability, antagonized the effect of nitro-L-**arginine**, as did L-**arginine**. D-1 receptor antagonists impaired both learning and memory, and L-**arginine** rendered learning values normal. The dopamine and D-1 receptor-agonist effect on 24 h memory was concentration dependent, and their higher concentrations substantially increased the retention indexes. The intimate mechanisms of these interactions are to be identified in further experiments.

CC Behavioral Biology - Animal Behavior *07003
Biochemical Studies - Proteins, Peptides and Amino Acids *10064
Biophysics - Molecular Properties and Macromolecules *10506
Biophysics - Membrane Phenomena *10508
Enzymes - Physiological Studies *10808
Cardiovascular System - Physiology and Biochemistry *14504
Endocrine System - Neuroendocrinology *17020
Nervous System - Physiology and Biochemistry *20504
BC Muridae *86375
IT Major Concepts
Behavior; Biochemistry and Molecular Biophysics; Cardiovascular System (Transport and Circulation); Endocrine System (Chemical Coordination and Homeostasis); Enzymology (Biochemistry and Molecular Biophysics); Membranes (Cell Biology); Nervous System (Neural Coordination)
IT Chemicals & Biochemicals
NITRIC OXIDE; DOPAMINE; **NITRIC OXIDE** SYNTHASE
IT Miscellaneous Descriptors
BRAIN; DOPAMINE; D1 RECEPTOR; LEARNING; MEMORY; NERVOUS SYSTEM; NEWBORN; **NITRIC OXIDE**; **NITRIC OXIDE** SYNTHASE
ORGN Super Taxa
Muridae: Rodentia, Mammalia, Vertebrata, Chordata, Animalia
ORGN Organism Name
rat (Muridae)
ORGN Organism Superterms
animals; chordates; mammals; nonhuman mammals; nonhuman vertebrates; rodents; vertebrates
RN 10102-43-9 (**NITRIC OXIDE**)
51-61-6 (DOPAMINE)
125978-95-2 (**NITRIC OXIDE** SYNTHASE)

file